

Please delete the paragraph on page 7, lines 7-18, and replace it with the following paragraph:

“ORP150” or “ORP” as used herein refers to oxygen regulated protein 150 (ORP150) or a fragment of ORP150. The amino acid sequence for human ORP150 is provided in Figure 16 (NCBI database Accession AAC50947, Accession NP\_006380). The term ORP150 also includes portions of, variants of, or allelic variants thereof. In the disclosed invention, a fragment of ORP150 is a fragment of the ORP150 protein which has an amino acid sequence which is unique to ORP150. The fragment may be as few as 6 amino acids, although it may be 7, 8, 9, 10, 11, 12, 13, 14, 15 or more amino acids. In one embodiment, the fragment comprises or consists of the sequence LAVMSVDLGSESM (**SEQ ID NO: 2**). The fragment may have a molecular weight in the range of from 6 to 8, 6.5 to 7.5, 6.7 to 7.4, 1 to 4, 1.5 to 3.5, or 1.8 to 3.3 kD. The molecular weight may be determined by means known to those skilled in the art such as gel electrophoresis or size exclusion chromatography.

Please delete the paragraph on page 9, lines 22-26, and replace it with the following paragraph:

The immunoassay may be comprised of an antibody or portion thereof sufficient for binding specifically to ORP150. One antibody useful for detecting ORP150 may recognize the sequence LAVMSVDLGSESM (**SEQ ID NO: 2**). Other suitable antibodies are available commercially from Immuno-Biological Laboratories Co. Ltd, 1091-1 Naka, Fujioka-shi, Gunma, 375-0005, Japan.

Please delete the paragraph on page 16, line 11, to page 17, line 14, and replace it with the following paragraph:

A peptide corresponding to the N-terminal domain (amino acids 33-45) of the human ORP150 sequence (LAVMSVDLGSESM; **SEQ ID NO: 2**) (Ikeda, *et al*, *Biochem Biophys Res Commun* 1997; **230**: 94-9) was synthesized in the MRC Toxicology Unit, University of Leicester. Amino acids 1-32 may represent a signal sequence for the protein and may not be present in the mature ORP150 protein. A rabbit was injected monthly with this peptide conjugated to keyhole limpet hemocyanin using maleimide coupling to a cysteine added to the

C-terminal of the sequence. IgG from the sera was purified on protein A sepharose columns. The above peptide was also biotinylated using biotin-maleimide in buffer containing (in mmol/l)  $\text{NaH}_2\text{PO}_4$  100, EDTA 5, pH 7.0 for 2 hours. After quenching with excess cysteine, the tracer was purified on HPLC using an acetonitrile gradient. Alternatively, the above peptide could be synthesized with incorporation of a biotinylated amino acid at the C- or N-terminus and used as a tracer. Plasma extracts and standards were reconstituted with ILMA (immunoluminometric assay) buffer consisting of (in mmol/l)  $\text{NaH}_2\text{PO}_4$  1.5,  $\text{Na}_2\text{HPO}_4$  8, NaCl 140, EDTA 1 and (in g/l) bovine serum albumin 1, azide 0.1. ELISA plates were coated with 100 ng of anti-rabbit IgG (Sigma Chemical Co., Poole, UK) in 100  $\mu\text{l}$  of 0.1 mol/l sodium bicarbonate buffer, pH 9.6. Wells were then blocked with 0.5% bovine serum albumin in bicarbonate buffer. A competitive immunoluminometric assay was set up by preincubating 200 ng of the IgG with standards or samples within the wells. After overnight incubation, 50  $\mu\text{l}$  of the diluted biotinylated ORP peptide tracer (2  $\mu\text{l}$  /ml of the stock solution or a total amount of 100-500 fmol) was added to the wells. Following another 24 h of incubation at 4°C, wells were washed 3 times with a wash buffer ( $\text{NaH}_2\text{PO}_4$  1.5 mmol/l,  $\text{Na}_2\text{HPO}_4$  8 mmol/l, NaCl 340 mmol/l, Tween 0.5 g/l, sodium azide 0.1 g/l). Streptavidin labeled with methyl-acridinium ester (MAE) was synthesized as described (Ng *et al*, *Clinical Science* 2002; **102**: 411-416). Wells were incubated for 2 h with 100  $\mu\text{l}$  of ILMA containing streptavidin-MAE (5 million relative light units per well). Following further washes, chemiluminescence was detected by sequential injections of 100  $\mu\text{L}$  of 0.1 M nitric acid (with  $\text{H}_2\text{O}_2$ ) and then 100  $\mu\text{L}$  of NaOH (with cetyl ammonium bromide) in a Dynatech MLX Luminometer. The lower limit of detection (defined as 3 times standard deviation at zero peptide concentration) was 9.8 fmol per tube or 98 fmol/ml of plasma extracted. Within assay coefficients of variation were 3.1, 4.3 and 5.9% for 2, 30, 500 fmol/tube respectively. There was no cross-reactivity with peptides previously demonstrated to be elevated in heart disease such as ANP, BNP, N terminal proBNP or CNP.

Please delete the paragraph on page 28, lines 5-11, and replace it with the following paragraph:

The cut-off values specified above are based on extracts of ORP150 from plasma, using peptide standards composed of CLAVMSVDLGSESM **(SEQ ID NO: 3)** where

LAVMSVDLGSESM (**SEQ ID NO: 2**) is derived from the N-terminal sequence of ORP150.

Due to the presence of the cysteine at the N-terminal (in order to produce the conjugates for immunisation in the first instance), there is a tendency for this peptide to form dimers. A variable proportion of dimers and monomers of the standard could lead to differences in immunoreactivity, and hence differences in actual cut-off values.

Please delete the paragraph on page 28, lines 12-24, and replace it with the following paragraph:

When an entire protein sequence is used as the standard (e.g. full length ORP150) or if the above peptide CLAVMSVDLGSESM (**SEQ ID NO: 3**) is reduced using dithiothreitol and reacted with N-ethylmaleimide to prevent dimer formation, it is likely that immunoreactivity for this epitope with the antibodies raised could be different, and hence cut-off values could be different. Correction factors of up to 10-100 times the above mentioned cut-offs may need to be applied for different standards or different assay formats (e.g. a non-competitive as opposed to a competitive format). However, it is likely that cut-off values would lie in the range 10-10,000 fmol/ml and each new assay may have its own cut-off values assigned to it for each specific purpose (diagnosis or prognosis), in order to apply it to the uses described in the examples. These cut-off values will also differ according to whether the test is used for diagnosis of heart failure, or estimating prognosis after myocardial infarction or unstable angina, as illustrated in the examples above.